Rec'd PCT/PTO 13 JUN 2001

FORM PTO-1390 (REV. 11-2000)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: 145865.00005

U S APPLICATION NO (If known, see 37 CFR 1 5: 09/868025

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IN00/00099	11 October 2000	13 October 1999

TITLE OF INVENTION ISOLATED NUCLEIC ACID SEQUENCE CONFERRING SALT TOLERANCE IN RICE PLANT

APPLICANT(S) FOR DO/EO/US Dr. Villo Morawala PATELL, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [x] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [x] is attached hereto (required only if not communicated by the International Bureau).
   - b. [ ] has been communicated by the International Bureau.
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. [ ] is attached hereto.
   - b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [x] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   - b. [ ] have been communicated by the International Bureau.
   - c. [x] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [x] Other items or information: Express Mailing Certificate, Return Postcard, Transmittal Letter (in duplicate), Check for basic filing fee of, $860, Preliminary Amendment, PCT Request Form PCT/RO/101, International Application No. WO 01/30990 A2, Application Specification, Letter dated 28 Feb 01 to WIPO, Letter dated 12 June 01 to WIPO, Second Letter dated 12 June 01 to WIPO, and Applicants' Redlined Specification showing changes.

JC03 Rec'd PCT/PTC 13 JUN 2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/868025	INTERNATIONAL APPLICATION NO PCT/IN00/00099	ATTORNEY'S DOCKET NUMBER 145865.00005

				CALCULATIONS	PTO USE ONLY
21. ☑ The following fees are submitted: **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... **$1000.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... **$860.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... **$710.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... **$690.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**					
ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	7 - 20 =		x **$18.00**	$	
Independent claims	1 - 3 =		x **$80.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +				$	
SUBTOTAL =				$ 860.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 860.00	
				Amount to be refunded:	$
				charged:	$

a. ☑ A check in the amount of $ 860.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. ______ in the amount of $ ______ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1682. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Powell, Goldstein, Frazer & Murphy LLP
P.O. Box 97223
Washington, DC 20090-7223
(202)347-0066

N Woodward Reg No. 35,020
SIGNATURE

for Richard Wilder
NAME

31,202
REGISTRATION NUMBER

June 13, 2001

#3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Villoo Morawala PATELL et al.	
U.S. Patent Application No. 09/868,025	Docket No.: 145865.00005
Filed: October 11, 2000	
For: ISOLATED NUCLEIC ACID SEQUENCE CONFERRING SALT TOLERANCE IN RICE PLANT	

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This is in response to a Communication from the Office, and a Notice to Comply with the Sequence Listing Requirements, both dated August 17, 2001, requesting a submission of an initial computer readable form (CRF) in compliance with the sequence listing disclosure rules of 37 C.F.R. §§1.821-1.825.

IN THE SPECIFICATION:

Please replace page 17, entitled "Sequence Listings" with the attached substitute paper copy of the Sequence Listing.

REMARKS

Applicant submits this Preliminary Amendment to provide as a separate part of the disclosure, a replacement "Sequence Listing" to comply with the sequence listing disclosure rules of 37 C.F.R. §§1.821-1.825.

Applicant submits that the substitute paper copy of the Sequence Listing is supported in the application as filed on October 11, 2000, and that the substitute Sequence Listing does not include new matter. Applicant also provides herewith in paper copy and on floppy disk the replacement Sequence Listing in computer readable form.

Applicant respectfully requests entry of this Amendment prior to examination of the present application.

Respectfully submitted,

Richard Wilder
Reg. No. 31,202

POWELL, GOLDSTEIN, FRAZER & MURPHY, LLP
P.O. Box 97233
Washington, D.C. 20090-7223
202-347-0066

Date: **February 19, 2002**
RW/BM/DWW/yea

::ODMA\PCDOCS\WSH\255504\1

PTO 19 FEB 2002

#3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Villoo Morawala PATELL et al.	**ATTN: APPLICATION DIVISION**
Serial No. 09/868,025	Docket No.: 145865.00005
Filed: October 11, 2000	
For: ISOLATED NUCLEIC ACID SEQUENCE CONFERING SALT TOLERANCE IN RICE PLANT	

<u>STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 C.F.R. §§ 1.821 – 1.825</u>

Box Missing Parts
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby state that the information recorded in computer readable form is identical to the substitute paper copy of the Sequence Listing submitted herewith.

I hereby state that the substitute paper copy of the Sequence Listing is supported in the application as filed on October 11, 2000 and that the substitute Sequence Listing does not include new matter.

Respectfully submitted,

Richard Wilder
Reg. No. 31,202

RW/BM/DW/yea
Date: February 19, 2002
Powell, Goldstein, Frazer & Murphy, LLP
P.O. Box 97223
Washington, D.C. 20090-7223

#3

SEQUENCE LISTING

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        Patell, Villoo M.
        Antony, Mathai C.
        Chandran, Divya
        Madurappa, Ashok

<120> Isolated Nucleic Acid Sequence Conferring Salt Tolerance in Rice Pla
nt

<130> 145865.00005

<140> US 09/868025
<141> 2000-10-11

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His Arg Glu Arg Ile Pro Cys Leu Ala Leu Gly Val Thr Ala Ile Cys
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Cys Leu Thr Asn Asn Gly Gly Cys Tyr Gly Glu Cys His Gly Ser Leu
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Gly His Val Asp Arg Phe Pro Gln His Ser Asn Glu Trp Asn Ser Gly
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ENTERED

PCT09

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                    RAW SEQUENCE LISTING                      DATE: 03/22/2002
                    PATENT APPLICATION:  US/09/868,025        TIME: 11:42:02

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      4       Patell, Villoo M.
      5       Antony, Mathai C.
      6       Chandran, Divya
      7       Madurappa, Ashok
      9 <120> TITLE OF INVENTION: Isolated Nucleic Acid Sequence Conferring Salt Tolerance in
Rice Plant
     11 <130> FILE REFERENCE: 145865.00005
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RAW SEQUENCE LISTING DATE: 03/22/2002
PATENT APPLICATION: **US/09/868,025** TIME: 11:42:02

Input Set : **A:\145865.00005 (Patel) .ST25.txt**
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VERIFICATION SUMMARY
PATENT APPLICATION: **US/09/868,025**

DATE: 03/22/2002
TIME: 11:42:03

Input Set : **A:\145865.00005 (Patel) .ST25.txt**
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L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date

09/868025
JC03 Rec'd PCT/PTO 13 JUN 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 145865.00005

Group Art Unit: Not yet assigned Examiner: Not yet assigned Inventor: PATELL, et al. Serial No. Not yet assigned Based on PCT/IN00/00099 filed 11 October 2000 Filed: June 13, 2001 For: ISOLATED NUCLEIC ACID SEQUENCE CONFERRING SALT TOLERANCE IN RICE PLANT	**PRELIMINARY** **AMENDMENT**

Box PCT
Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Please amend the application as follows:

IN THE SPECIFICATION:

On page 8, delete the paragraph beginning on line 15, which reads:

[The prior art for our experiments includes interalia the work done by scientists in relation to rice and proteinase inhibitors discovered from rice under biotic conditions.]

On page 8, delete the paragraph beginning on line 19, which reads:

[Also the prior art known in this field addressed only biotic stress (host-pathogen interaction), whereas our invention addresses a novel issue that is the area of abiotic stress (salinity stress).]

On page 9, the paragraph beginning on line 1 is rewritten to read:

The polypeptide has glycosylation and phosphorylation sites. The said glycosylation is O glycosylation.

On page 9, delete the paragraph beginning on line 8, which reads:

[Said polypeptide has similarity with proteinase inhibitors of Bowman Birk type II of super family of proteinase inhibitors.]

On page 9, delete the paragraph beginning on line 16, which reads:

[The invention has use over a broad range of types of plants and organisms. Such plants *interalia* includes cotton, maize, rice, soybeans, sugar beet, wheat, fruit, vegetables and vines. The major use of proteinase inhibitors is against biotic stress response such as bacterial, fungal, pest resistance etc. in plants. It is also useful in the treatment of cancer, HIV and other areas in the animal systems. The gene may be useful for food processing and enzyme industries as an inhibitor of proteinase activity as a biological preservative.]

On page 11, the paragraph beginning on line 29 is rewritten to read:

The structure and function of AGT-SAL-11 was predicted using computational Biology, (Bioinformatics). Bioinformatics is a theoretical approach where predictions are carried out using computer applications; the Biological Data generated from the Laboratories till date is the source for the information on which the entire analysis was based.

On page 12, delete the paragraphs beginning on lines 21, 24, 26, 30, and 35, which read:

[AGT-SAL-11 molecule shows similarity with Proteinase Inhibitors of the Bowman – Birk II type of super-family of Proteinase Inhibitors, which are from the following species.]

[Ex. *Vicia faba, Vigna sp., Glycine max (Soyabean)* .]

[These molecules are generally bi-functional units, which can act on two different substrates. (Substrates being Chymotrypsin , Elastase, Trypsin, subtilisin) .]

[These Bowman –Birk type Proteinase inhibitors including AGT-SAL-11 molecules commonly have Glycosylation sites where a carbohydrate moiety can bind, most likely carbohydrates which bind with these molecules are Mannose sugars.]

[The 3D Structure of the Bowman–Birk type proteinase inhibitors shows the molecules tend to have an αβ type of folding.]

On page 12, the paragraph beginning on line 38 is rewritten to read:

The Secondary structure of AGTSAL-11 was predicted using the applications of Predict Protein Server. The results obtained are as

On Page 13, the paragraph beginning on line 1 is rewritten to read:

a) The molecule shows a mixture of $\alpha\beta$ type of secondary structure.

b) There are sites for Glycosylation and Phosphorylation (mostly Oglycosylation with Serine or Threonine residues).

On Page 13, delete the paragraph beginning on line 3, which reads:

Inhibitors of the Bowman Birk type are relatively small (about 70 amino acids length) and multiply cross linked with disulfide bridges. The Bowman- Birk inhibitors often display dual specificity, inhibiting both trypsin and chymotrypsin. No pattern has emerged to establish which inhibitors have protective effect and which do not. Inhibitor specificity does not appear to be the only factor, since some trypsin inhibitor are effective while others are not.

<u>**IN THE CLAIMS**</u>

Delete original claims 3, 4, 5 and 9.

REMARKS

Entry of the above amendments prior to examination is respectfully requested. Attached hereto is a redline copy of the application showing changes made to the specification by the current amendment.

Respectfully submitted,

[illegible]

For Richard Wilder
Registration No. 31,201
Attorney for Applicant

POWELL, GOLDSTEIN, FRAZER & MURPHY LLP
P.O. Box 97223
Washington, D.C. 20090-7223
(202) 347-0066

Dated: June 13, 2001

RW/db

09/868025

21 parts

PCT/IN00/00099
JC03 Rec'd PCT/PTC 13 JUN 2001

Isolated nucleic acid sequence conferring salt tolerance in rice plant

Field of the invention

The present invention relates to an isolated nucleic acid sequence conferring salt tolerance in rice plant. More specifically this invention relates to a method for conferring salt tolerance in plants.

Altered gene expression lies at the heart of regulatory mechanisms that control cell biology. Comparisons of gene expression in different cell types provide the underlying information that analyzes the biological processes that control our lives. Effective methods are needed to identify and isolate those genes that are differentially expresses in various cells or under altered conditions.

Background

Life can not exist without water. It forms an important constituent of the plant and animal cell and is present to the extent of 80 to 90%. Water is essential for plants due to the following reasons :

1. It is the major component of protoplasm. If the protoplasm is dehydrated, it ceases to be active and the protoplasm looses its physical and chemical properties. Water maintains turgidity of cells.

2. Water is a universal solvent. The intake of minerals and nutrients from the external medium into the cell is only in the dissolved form.

3. Water serves as the medium for translocation of minerals from the soil to leaves through the xylem and food manufactured by the leaves to other plant parts via phloem.

4. Water also plays an important role in the transport of plant hormones.

5. Plant movements (especially of certain organs) are caused by changes in water content of cells.

6. Water is directly involved in the bio-chemical reactions that take place in plant cells. Hydrolyis of macromolecules takes place by the addition of water. Water is the source of hydrogen for the reduction of carbon-dioxide during photosynthesis. Water is one of the products of cellular respiration. All these reactions are influenced by the availability of adequate and good quality water.

Since water plays such an important role in plants, its deficit severely effects cellular functions, plant growth & development and reduces yields . However the plant devises a number of changes that occur at the whole plant, physiological, cellular, bio-chemical and molecular levels in an attempt to cope with moisture stress.

Furthermore, due to the widespread use of irrigation and limited water supply, many cultivated areas have become increasingly salinized. Irrigation imparts increasingly salt concentration when the available irrigation water evaporates and leaves previously dissolved salts behind.

Dissolved salts in the soil increase the osmotic pressure of the solution in the soil and tends to decrease the rate at which water from the soil will enter the roots. If the solution in the soil becomes too saturated with dissolved salts, the water may actually be withdrawn from plant roots. Thus the plants slowly starve though the supply of salts and dissolved nutrients may be more than ample.

Salinity and water deficit have shown to induce the expression of number of genes. These gene products have either regulatory role in gene expression or a functional role in adaptive responses of plant cells to the stress.

Salinity refers to the presence of various salts in soil and irrigation water in concentrations that affect the growth and yield of plants. Sodium chloride (common salt), is often the dominant salt present in saline soils. Saline-alkaline and sodic soils may have excess of chlorides, sulphates and bicarbonates of sodium, calcium and potassium in addition to other inorganic ions.

Saline soils have a soil water conductivity of 4 deci-seimen/meter and exchangeable sodium percentage of not less that 15. This translates into nearly 2.56 g/L of total dissolved salts in an extract or if all the salt is NaCl, an ionic concentration of 44.14 mM.

The irrigation water in majority of the rice growing areas is generally of marginal or poor quality (EC of 2-5ds/m or more). Though water is present it is unavailable to plants because the osmotic potential of soil is altered. To exclude salts and minimize ion toxicity, water must be imported against a free energy gradient. However, if water is taken up freely, the endogenous salt concentration rises.

Macromolecular assemble and enzyme activity associated with shaping and maintaining each cell can proceed only with a properly constituted ionic environment. The inorganic ions selectively neutralize charges on macromolecular surfaces and simultaneously permit formation of intramolecular bridges that determine the final conformation of many proteins. The same ions also determine the availability of free water around enzymes and their substrates and thus the rate of catalysis. Finally, ionic gradients, set up at considerable cost to the plant cell, constitute free energy gradients that can be tapped to direct the flow of organic molecules and between cells [Claes et al., 90].

An extracellular ion excess invariably disrupts the ionic balance intracellularly. With the influx of salt, proteins may denature or aggregate leading to a loss of function, gradient-driven pumps may reverse and thus block the normal redistribution of symported molecules , membrane fluidity and consequently, the activity of some membrane components may change, and even the entry of water may be restricted . Some ions may have additional secondary effects. For example, increasing amounts of intercellular Na+ can lead to decreases in the concentration of K+ [Ben-Hayyim et al., 1987; Binzel et al., 1988]. This, in turn, reduces the rate of photosynthesis[Pier and Berkowitz, 1987], and , based on studies with bacteria can accelerate polysome decay and degradation of the free ribosomal proteins [St. John and Goldberg, 1980]. Salt imposed stress has been shown to have an impact even before ions enter the cell. Extracellular Na+ (or mannitol), for example, can leach Ca^{2+} from root cell plasmalemma, and as a result of membrane destabilization, increases K+ efflux[Cramer et al., 1985].

These are only the immediate problems facing the cell. If the stress is prolonged, normal maintenance processes are impaired because general protein synthesis [Hurkman and Tanaka, 1987] and metabolism [Criddle et al., 1989] both decline. Denatured proteins may form inactive complexes with otherwise functional proteins. Enzymes may be poisoned when inorganic cofactors are displaced by incoming salts.

Rice is a salt sensitive plant and the most important cereal crop of the world. This crop is grown in diverse ecosystems and extensively in the tropics. Rice is the staple food of majority of the people in South & East Asian nations, parts of South America and Africa. The present production of rice in the world falls well short of the demand. To meet the ever-increasing demand, continuous improvement in the quality and productivity of this cereal is vital.

Several biotic and abiotic factors are important constraints in increasing the quality and yield of rice. Biotic stresses in the form of pests and diseases considerably affect rice productivity. Abiotic stresses however have been shown to cause more harm to the rice crop than biotic stresses. The major abiotic stresses, which significantly hamper rice yields are drought, salinity, floods, extremes of temperature and metal toxicity.

Minimizing crop losses by abiotic stresses especially drought and salinity is an important area for the overall improvement of rice. A thorough understanding of the responses of the rice plant to abiotic stresses is fundamental for developing a strategy to make the rice plant more hardy.

Initial work in understanding the effects of abiotic stress on rice was done at the whole plant level. The role, interactions and alterations of root-shoot characteristics in response to stress in rice has been studied. Later work focused on the physiology of stress. The effects of drought and salinity on the physiological processes like metabolism, growth and development has come forth.

Efforts have also been made to improve the performance of rice crop under limiting environmental conditions through traditional breeding programs and agronomic practices. Strategies for the evaluation of rice for drought and salinity tolerance using field screening and multi-location testing have been developed. These approaches have also been able to distinguish rice varieties into susceptible and tolerant ones. The development of molecular linkage maps and the use of molecular markers, of late, is helping selection and breeding for drought resistance. Molecular markers linked to root traits, osmotic adjustment and other stress tolerant characters are now being identified and used for selection and breeding.

The molecular responses of plants to abiotic stresses is a complex phenomenon. However, advances in molecular biology offer new tools to

investigate changes in plants, at the cellular and molecular level, in response to abiotic stresses.

Relatively speaking, this species is more sensitive to salt stress at the seedling stage and the reproductive stage [Lutts et al., 1995]. Excess salt leads to reduced seed germination and poor seedling vigor. During the vegetative phase, premature senescence of leaves and reduced number of tillers can occur. During the reproductive stage, the number of spikelets per panicle get significantly reduced [Lutts et al., 1996]

Furthermore, rice cultivation in tropical areas is mostly dependent on seasonal rainfall, vagaries of tropical monsoon renders the growth and yield of rice crop uncertain. The modern high yielding varieties of rice in particular are unable to attain their full genetic potential in the absence of adequate and good quality water.

Drought occurs when there is insufficient soil water to be taken up by the plants over a period of time to meet its transpirational requirements. Sustained drought results in complete loss of free water and will result in desiccation and dehydration. Concentration of solutes in the cell leads to drop in cellular water potential. Loss of turgor leads to changes in the cell volume and membrane area. The crucial cell wall plasma membrane continuum is lost. An osmotic shock can cause extensive cell damage through disruption of membrane integrity and leakage of cellular contents. Cellular water deficit causes extensive damage to functional proteins and increases formation of misformed proteins. Impairment in the normal metabolic pathways leads to formation of toxic and highly reactive by products such as the reactive oxygen species. Many other cellular changes similar to those occuring during salt stress are also observed during drought.

In Rice, at the plant level, drought affects several developmental processes. Seed germination is non-uniform. At the Vegetative stage, canopy photosynthetic rates decrease drastically. Root growth is affected. Leaf rolling and leaf scorching is observed. At the reproductive stage, drought causes pollen sterility, small, thin and deformed anthers. Drought during anthesis causes inhibition of another dehiscence and pollen germination, reduced pollen viability, failure of the panicle to exert the flag leaf, resulting in loss of grain set. Water constraint during ripening causes incomplete grain filling [O'Toole and Moya, 1981].

Molecular Responses of Rice to Salinity and Moisture stress.

Osmotic stress (such as salinity and Drought) leading to water deficit elicit complex molecular responses in plants. The events described here are common to all plants and also apply to Rice.

The molecular responses of plants to water deficit is dependent upon the type of stress(salinity/drought), severity of stress (mild/moderate or severe) and duration of stress (sporadic or chronic). A gradual onset of stress allows cellular mechanisms to adopt better while a sudden severe stress results in cellular damage and activates repair mechanisms. Plant factors such as genotype/variety, developmental stage (seed/seedling/vegetative or reproductive stage) and organ (root/shoot etc.) exposed to stress also influences the nature of response [Bray, 1997].

Molecular events during water deficit has been investigated using four major approaches [reviewed in Ingram and Bartels, 1996] :

1. Examining tolerant systems such as seeds and resurrection plants.
2. Analyzing mutants from genetic model species.
3. Analyzing the effects on agriculturally relevant plants.
4. By the targetted expression of drought related genes in vivo using transgenic plants.

The responses of plants to water deficit at the molecular level normally occur in the following sequence [Bray, 1993}"

1. Cellular perception of the stress.
2. Signal transduction events.
3. Alterations in the gene expression.
4. The role of gene products induced by salinity and drought in stress avoidance of tolerance.

The Role of Gene Products induced by Salinity and Drought.

Salinity and Water deficit have shown to induce the expression of a number genes. These gene products have either a regulatory role in gene expression or a functional role in the adaptive responses of plant cells to the stress.

Many genes have been identified and characterized to have a definite role in the response of plants to salinity and drought, and are induced by a complex mechanism of stress perception and signal transduction events. Stress related gene products have a role in moisture stress tolerance such as signaling molecules, regulatory proteins, protection of cellular structures, synthesis of osmoprotectants, ion sequestration, chaperon activity and protein stabilization, protein degradation, scavenging of accumulated toxins(especially reactive oxygen species), promotion of damage repair mechanisms, anti-pathogen activity and others.

Changes, in the tissue specific gene expression, are fundamental to the responses that occur during salinity and drought and influence many of the short and long term cellular changes that determine stress resistance or susceptibility. Northern Blot analysis, using stress related cDNA probes, offers a simple but powerful tool to monitor alterations in gene expression in roots and shoots, in response to salinity and water deficit, while comparing a susceptible and tolerant variety.

Furthermore, subtractive hybridization technique has been used for identifying and cloning differentially expresses mRNAs. The basic principle of subtractive hybridization involves the hybridization of cDNAs from one population in which mRNAs are differentially expressed to excess constitutively expressed cDNAs from another population. The sequence that are common to both the populations are removed using hydroxypatite chromatography, avidin biotin binding or oligo- dT beads. Despite the enormous success of subtractive techniques in cloning different genes, this requires multiple subtraction steps. Therefore, a new strategy was developed which permits exponential amplification of cDNAs that differ in abundance in 2 populations is suppressed.

Differential display is also a power tool for analyzing gene expression, allowing genes to be isolated solely on changes in phenotype and without prior knowledge of protein or nucleic acid sequence. This technique is flexible and is a comprehensive method for detecting almost all genes expressed in a particular cell and for identification of differences in gene expression between different cell types in both mammal and plant systems. There is a simultaneous display of up and down regulated genes, it permits side-by –side comparisons of mRNA from different sources; only a few (g of RNA is required, compared to 50X or more for subtractive hybridization; highly reproducible and finally high speed of analysis.

This method involves the reverse transcription of the mRNA with oligo-dT primers anchored to the beginning of the poly (A) tail, followed by the polymerase chain reaction on the presence of a second 10mer, arbitrary in sequence. PCR primers and conditions are chosen such that any given reaction yields a limited number of amplified cDNA fragments permitting their visualization as discrete bands following Gel Electrophoresis. The amplified cDNA sub-populations of 3' termini of mRNAs as defined by this pair of primers are distributed on a DNA sequencing gel and visualized by autoradiography. Each pair of the primer produces a distinct pattern of bands. The band pattern obtained with each primer is compared. Differentially expressed bands are cut out of the gel and the DNA is eluted and re-amplified. The amplified products are cloned into suitable vectors and their sequence deduced.

The prior art for our experiments includes interalia the work done by scientists in relation to rice and proteinase inhibitors discovered from rice under biotic conditions.

Also the prior art known in this field addressed only biotic stress (host-pathogen interaction), whereas our invention addresses a novel issue that is the area of abiotic stress(salinity stress).

Summary of the invention

The object of the present invention is to correlate the expression pattern (at the mRNA levels) of genes under study with their role in abiotic stress tolerance or susceptibility in IR64 (susceptible variety) and RASI (tolerant variety).

Yet another object of the present invention is to compare the differences in the expression of genes encoding stress proteins during salinity and desiccation.

Further object of this invention is to assess the gene expression pattern in root and shoot during different stages of salt and dehydration.

To achieve the said objects, the present invention relates to relates to a nucleic acid sequence comprising a polynucleotide, AGT- SAL 11 having a sequence SEQ.ID No.1.

The AGT-SAL11 polynucleotide sequence encodes a polypeptide as shown in SEQ ID No. 2.

The polynucleotide sequence is a full length AGTSAL 11 gene. The said polypeptide has bi-functional units.The said polypeptide has glycosylation and phosphorylation sites. Said glycosylation is O glycosylation.

Said AGT-SAL 11 has a mixture of $\alpha\beta$ type of secondary structure.

Said polypeptide has similarity with proteinase inhibitors of Bowman Birk type II of super family of proteinase inhibitors.

The present invention further relates to a method for conferring salt tolerance on a plant, the method comprising introducing into the plant a recombinant expression cassette comprising a plant operator operably linked to AGT-SAL 11 polynucleotide sequence.

The invention has use over a broad range of types of plants and organisms. Such plants *interalia* includes cotton, maize, rice, soybeans, sugar beet, wheat, fruit, vegetables and vines. The major use of proteinase inhibitors is against biotic stress response such as bacterial, fungal, pest resistance etc. in plants. It is also useful in the treatment of cancer, HIV and other areas in the animal systems. The gene may be useful for food processing and enzyme industries as an inhibitor of proteinase activity as a biological preservative.

Detailed description of the invention

Two Indian varieties of rice IR64 and RASI were taken. While IR-64 is susceptible to high salt stress, RASI is resistant to the same. The differential display technique was used to determine the regulation of gene expression at the cellular level in these two varieties under salt stress conditions and isolate the genes responsible for susceptibility or resistance in IR-64 and RASI respectively.

IR-64 and RASI seeds were subjected to salt stress using 150mM NaCl. The RNA was isolated from both stressed plants and unstressed controls. Further processing of the RNA was done following the protocol provided by Gen-Hunter's differential display kit. The RNA was reverse transcribed using H-T11 primers to obtain the cDNA. This DNA was amplified by PCR using H-T11 primers and an arbitrary primer H-API. The PCR products were resolved on a 6 % denaturing polyacrylamide gel and subjected to autoradiography. The autoradiogram showed 54 differentially expressed bands. The band labeled A-11

was cut out from the gel and DNA eluted. Reamplification of the DNA was done using the same primer set and PCR conditions.

The PCR product of AGT-SAL was cloned into TOP TA cloning vector, which is a unique, fast and an efficient way to clone PCR products. The vectors are linearized having an extra 3'T overhang and are activated with topoisomerase. Ligation takes advantage of the template independent addition of a single adenosine (A) to the 3' end of the PCR products by Taq DNA Polymerase. The positive clones were checked for the presence of insert by digesting with EcoRI restriction endonuclease.

Two clones showing insert release were subjected to sequencing using sequences of the vector that flank the insert sites as primers; M13 forward and reverse primers shows that the sequence of interest lies between nucleotides 130 and 310 which ends with a stretch of poly A's. Since the fragment of interest was amplified using specific oligo-dT primers, its position in the sequence was located by searching for a poly A stretch downstream. This stretch was found around position 310, indicating the 3' end of the sequence of interest.

For expressing the vector, the gene AGTSAL-11 (Accession No. AF 192975) should be cloned in expression vector where the protein of interest would be induced under inductive condition. There are so many vectors being used for this purpose, which ideally contain artificial ribosome binding site, transcription start site, transcription terminator, inducible promoter and a multiple cloning site (MCS) for cloning of desired gene at a particular site and a module for purification of he protein in the induced state. For the purification of protein of interest under inducible condition there are several criteria that can be used such as GST (Glutathion S transferase) fusion protein where protein of interest can be purified by Glutathion affinity column and further the protein can be obtained by the treatment of endopeptidase with GST peptide specificity. The other popular protein expression has 6 X His tag which is coded by the sequence prior to the gene of interest, has affinity with Ni-affinity column and the protein of interest can be purified by imidazole elution. The pQE vectors (commercially available from Quigen) can be used for cloning the gene in three different frames such as 0, -2 and –1 frame(pQE-30, pQE-31 and pQE-32).

For this cloning, the AGT-SAL gene was first cloned in pBSKS(+) at EcoRI site (as a vector) whereas the gene was obtained from pTAdv-Sal and transformed to DH10B competent cells. The transformants were selected on LB Agar Amp(-IPTG/X-gal-) and white colonies were screened for the presence of

insert using EcoRI and KpnI/SacI. The orientation of the insert was analysed using enzymes such as PstI, NcoI-SacI etc. The construct was named as pSV-SAL. From pSV-SAL, the gene was directionally cloned into pQE (pQE-30, pQE-31 and pQE-32) vectors by using AGT-SAL KpnI/SacI double digest and transformed in DH10B competent cells. The transformants were selected on LB Agar (Amp) and the transformants were screened. The recombinants were confirmed by digesting transformants plasmid with EcoRI and the three constructs were named as pExSV(1)SAL (have backbone of pQE-30), pExSV(2)SAL (have backbone of pQE-31) and pExSV(3)SAL (have backbone of pQE-32).

Further all constructs were transformed to M15 (commercially available from Quigen) competent cells for expression. M15 cells are specifically expression cells because of the presence of pREP4 which overproduces Lac repressor protein for Lac promoter and so the induction of gene of interest is tightly regulated.

The M15 cells with three constructs were grown till it reached to log phase, induction with IPTG was given and allowed for 3-4 hours. The cells were pelleted and dissolved in Tris-phosphate urea buffer(pH8.0). The samples of these were loaded to acrylamide gel with uninduced sample as control. After the protocol is standardized it will be deduced as which one of them is expressing the protein under induced conditions. The native AGT-SAL is purified. The protein was purified by Ni-NTA affinity column which has affinity for the 6X His tag and the elution was performed by buffer containing imidazole which has higher affinity for Ni matrix and then in turn compete with 6X His tagged protein and replaces them.

The structure and function of AGT-SAL-11 was predicted using computational Biology, (Bioinformatics). Bioinformatics is a theoretical approach where predictions are carried out using computer applications; the Biological Data generated from the Laboratories till date is the source for the Databases.

Although in most cases protein production is the ultimate output for the gene protein analysis techniques are currently less suitable for high throughput screening than Nucleic Acid analysis techniques. Thus RNA analysis are the most important at present.

To find any similar pattern or similar molecules in the database a program BLAST (www.ncbi.nlm.nih.gov/BLAST) was performed but no significant results were obtained (using the gene sequence).

The subsequent tests mentioned below were performed to study the Protein level, the stage that actually determines the Function of a gene. (AAF06789.1)

The protein sequence was also subjected to similarity search initially with BLAST with BLOSUM -62, matrix but found no interesting results. BLOSUM stands for Block Summation matrix, which is used to find molecules, which are related to one another having similar sequences and accounts for similar functions as well.

For a more specific approach the tests were extended to FASTA, (www.ebi.ac.uk) a stringent method for finding sequence similarity, in this attempt we could count on a group of hits which resembled AGT-SAL-11, using a matrix of BLOSUM-40. BLOSUM 40 is used to find distantly related molecules.

AGT-SAL-11 molecule shows similarity with Proteinase Inhibitors of the Bowman – Birk II type of super-family of Proteinase Inhibitors, which are from the following species.

Ex. *Vicia faba, Vigna sp., Glycine max (Soyabean)* .

These molecules are generally bi-functional units, which can act on two different substrates. (Substrates being Chymotrypsin , Elastase, Trypsin, subtilisin) .

These Bowman –Birk type Proteinase inhibitors including AGT-SAL-11 molecules commonly have Glycosylation sites where a carbohydrate moiety can bind, most likely carbohydrates which bind with these molecules are Mannose sugars.

The 3D Structure of the Bowman–Birk type proteinase inhibitors shows the molecules tend to have an αβ type of folding.

The Secondary structure of AGT-SAL –11 was predicted using the applications of Predict Protein server. The results obtained are as

a) The molecule shows a mixture of αβ type of secondary structure.

b) There are sites for Glycosylation and Phosphorylation (mostly O Glycosylation with Serine or Threonine residues).

Inhibitors of the Bowman Birk type are relatively small (about 70 amino acids length) and multiply cross linked with disulfide bridges. The Bowman- Birk inhibitors often display dual specificity, inhibiting both trypsin and chymotrypsin. No pattern has emerged to establish which inhibitors have protective effect and which do not. Inhibitor specificity does not appear to be the only factor, since some trypsin inhibitor are effective while others are not.

Experimental Procedures

1. Collection of plant materials.

a. Seeds of IR64 and Rasi, the two varieties of *Indica* rice chosen for the study were dehusked and good seeds were selected. These were surface sterilized using 70 % ethyl alcohol for 1 minute and 2 % sodium hypochlorite for 20 minutes. Surface sterilized seeds were washed repeatedly with sterile distilled water to remove traces of sterilizing agents.

Circular sterile filter papers were placed in autoclaved plastic petriplates and moistened with 20 ml sterile distilled water in the laminar flow hood. About 25 surface seeds were placed in each plate and the lid was covered and the plates were incubated at room temperature.

The seeds on an average took 2 days for germination. After germination the seedlings were allowed to grow for one week. The plates were constantly monitored for contamination. Since the plant material was to be used for RNA extraction, plates with any sign of contamination was discarded. Petriplates were irrigated whenever necessary.

Nine day old seedlings were used for inducing salt and dehydration stresses.

b. Induction of Salt Stress

For the induction of salt stress, the water in the petriplates containing 9 day old seedlings was replaced with 150 mM NaCl solution. One, two, four, eight and sixteen hours were collected by excising the endosperm and separting the seedling into root and shoot. The plant material was immediately frozen in liquid nitrogen and stored at –80 degrees Celsius for RNA isolation later on.

c. Induction of moisture stress

Moisture stress was induced by allowing nine days old seedlings to desiccate gradually in inflated plastic bags at room temperature. Loss of weight of the seedling was constantly monitored. Plastic bags were changed frequently to decrease humidity inside the bag. When the seedlings recorded 30 % and 40 % weight loss, samples were collected by excising the endosperm and separating the seedling into root and shoot and freezing them.

d. Controls

Unstressed nine day old seedlings of Rasi and IR 64, collected in the same manner as described above, were used as controls.

II Isolation of Total RNA

a. Preparation of RNA extraction

The following precaution were taken to inhibit ribonucleases.

1. All glassware and heat resistant materials (pestle and mortar, forces etc. were baked overnight in an oven.
2. 0.1 % DEPC (diethylpyrocarbonate) was added to all solutions (except those containing Tris) incubated overnight after thorough shaking and then autoclaved.
3. All plastic ware were treated with 10 % hydrogen peroxide overnight, autoclaved and dried properly after use.
4. Clean disposable gloves were used at all stages of RNA extraction.

b. RNA Isolation

Single step method of RNA isolation by acid Guanidium thiocyanate Phenol- chloroform extraction (Sacchi et. Al 1987) was employed to isolate total RNA. The procedure consisted of following steps:

1. 0.5 to 1 gm of tissue was ground in liquid nitrogen using pestle and mortar to make a fine powder.
2. To this 6ml of freshly prepared extraction buffer was added and homogenized.
3. To the homogenate taken in a centrifuge tube, the following reagents were sequentially added and mixed thoroughly after addition of each reagent:
    a. ml of 2M sodium acetate (pH4)
    b. 10ml of phenol (Saturated with DEPC treated water)

c. 2ml of chloroform: isoamyl alcohol (49:1) mixture

This was incubated on ice for 15minutes and centrifuged at 8000 rpm for 12 minutes at 4 degrees C. The aqueous phase was carefully transferred to a fresh centrifuge tube and 10 ml of iso-propanol was added and mixed well and incubated at –20 degrees for 1 hour. The tube was centrifuged at 14,500 rpm for 20minutes at 4 °C. The pellet was re-suspended in 3ml of extraction buffer and 3ml of iso-propanol was added, mixed well and incubated at –20 °C for 1 hour. The tube was centrifuged at 14,500 rpm for 20 minutes at 4 °C. The pellet was washed with 1ml of 75 %ethanol, centrifuged at 14,500 rpm for 15minuted at 4 °C. The pellet was dissolved in DEPC treated water and stored at - 80°C.

a. Determination of RNA concentration

3µl of RNA extract was taken in 1ml of DEPC treated water for spectrophotometric quantification and purity analysis. Absorbance at 260nm and 280nm was taken using a "spectronic Genesis-5' spectrophotometer. RNA concentaruions were determined based on the relationship that an OD of 1 at 260nm corresponds to 40µg of RNA. RNA purity was assessed by calculating the A260/280 ratios (Table no. 1). The ratio should be close to 2 for a good RNA extraction.

b. Checking of RNA integrity by Submarine Agraose Gel electrophoresis.

A 100ml 1.2 % formaldehyde agarose gel was cast by melting 1.2g of agarose (RNase free) in 73.3 ml of DEPC treated water. Just before pouring the gel, 10 ml of 10 X MOPS/EDTA and 16.7 ml of formaldehyde (2.2M) was added.

30µg of RNA was taken in 25µl of the gel loading dye mixed well and heated at 65degrees celsius for 15 minute on a dry bath and snap cooled on ice before loading on the gel.

3µl of 0.24kb to 9.5kb RNA ladder from GIBCO BRL containing a mixture of 6 synthetic poly (A) tailed RNAs (0.5µg each) of sizes 9.49kb, 7.46kb, 4.40kb, 2.37kb, 1.35kb and 0.24kb was used as a marker for these gels (Fig no.2).

Horizontal or submarine agarose gel electrophoresis system was used. IX MOPS/EDTA was used as the electrode buffer. A potential difference of 5- 10 volts per cm (distance between the electrodes) was used for the anionic run.

The two prominent RNA bands of sized 4.7kb and 1.9kb correspond to 28s and 18s ribosomal RNA activity (figure no.1) Faint bands of 2.9kn (23s chloroplast rRNA) and 1.5kb (16s chloroplast rRNA) can also be visualized. 5s rRNA is about 120bp and runs faintly below the dye front. The 240bp RNA size marker comigrates with the Bromo-phenol blue dye front. The smear below the dye front also represents degraded RNA apart from tRNA and a small mRNA population. The rest of the RNA is the mRNA population. DNA(contamination) stays in the well hardly moves. A good RNA extract when runs on the gel shows minimum or no DNA in the well, distinct rRNA bands, prominent smear up to the dye front and a faint fizzy band below the dye front. (Fig No.1)

We claim:

1. An isolated nucleic acid sequence plant comprising a polynucleotide, AGT- SAL 11 having a sequence SEQ.ID No.1.

2. A nucleic acid sequence as claimed in claim 1 wherein said AGTSAL polynucleotide sequence encodes a polypeptide as shown in SEQ ID No. 2.

3. A nucleic acid sequence as claimed in claim 1 wherein said polynucleotide sequence is a full length AGTSAL 11 gene.

4. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide sequence is a complete and mature AGTSAL 11 protein.

5. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide has bi-functional units.

6. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide has glycosylation and phosphorylation sites.

7. A nucleic acid sequence as claimed in claim 2 wherein said glycosylation is O glycosylation.

8. A nucleic acid sequence as claimed in claim 2 wherein said AGT-SAL 11 has a mixture of $\alpha\beta$ type of secondary structure.

9. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide has similarity with proteinase inhibitors of Bowman Birk II type of super family of proteinase inhibitors.

10. A transgenic plant comprising a recombinant expression cassette comprising a plant promoter operably linked to the polynucleotide sequence as claimed in claim 1.

11. A method for conferring salt tolerance on a plant, the method comprising introducing into the plant a recombinant expression cassette comprising a plant operator operably linked to AGT-SAL polynucleotide sequence as claimed in claim 1.

SEQUENCE LISTINGS

SEQUENCE ID NO. 1

BASE COUNT 163 a 145 c 170 g 195 t
ORIGIN

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661 gtaaaaaaaa aaa
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SEQUENCE ID NO. 2:

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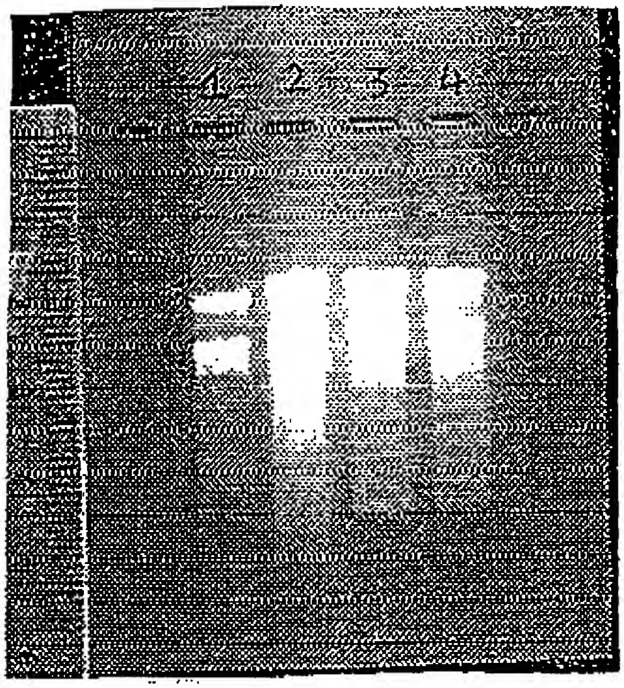
09/868025

WO 01/30990 PCT/IN00/00099



Lanes:	1	2	3	4
	IR64 S-S	IR64C	RA51 S-S	[illegible]
	10 µL	10 µL	10 µL	[illegible]

Fig. 1

09/868025

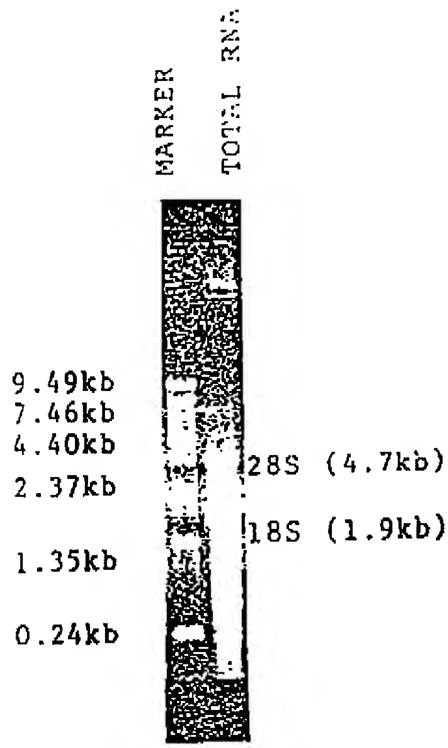


Fig.No.2: Sample RNA agarose (denaturing gel) with size markers.

100Rs.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the first inventor and joint inventor of the subject matter which is claimed and for which a patent is claimed and for which a patent is sought on the invention entitled

ISOLATED NUCLEIC ACID SEQUENCE CONFERRING SALT TOLERANCE IN RICE PLANT

The specification of which (check one)

[ ] is attached hereto.

[X] was filed on ___________ as Application Serial No. ____________ and was amended on ___________.

I hereby state that I have reviewed and understood the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty of disclosure information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, section 1.56.

100Rs.

DECLARATION AND POWER OF ATTORNEY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like somade are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey Kushan, Reg. No. 43,401, Thomas T. Moga, Reg. No. 34, 881, Richard Wilder, Reg. No. 31,202, David Fitzgerald, Reg. No. 43,347 and David W. Woodward, Reg. No. 35,020, and each principal, attorney of counsel, associate and employee of Powell, Goldstein, Frazer & Murphy, LLP, who is a registered Power of Attorney, my attorney with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. I request the Patent and Trademark Office to direct all correspondence and telephone calls relative to this application to Powell, Goldstein, Frazer & Murphy, LLP, P.O. Box 97223, Washington D.C. 20090-7223, (202) 347-0066.

1-60

Full name of sole or first inventor: Dr. Villoo Morawala Patell

Inventor's signature:

Date: 29/12/01

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Post Office Address: Same as residence

I hereby claim foreign priority benefits under Title 35, United States Code, section 119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS

			Priority Claim	
997/Mas/99	India	13^{th} October 1999	YES	
(Number)	(Country)	(Day/Month/Year filed)		
______	______	______	______	______
(Number)	(Country)	(Day/Month/Year filed)	Yes	No
______	______	______	______	______
(Number)	(Country)	(Day/Month/Year filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, ξ119(e) of any United States Provisional application(s) listed below:

PRIOR PROVISIONAL APPLICATIONS

______________________	______________________
(application serial number)	(Month/ Day/ Year filed)
______________________	______________________
(application serial number)	(Month/ Day/ Year filed)

I hereby claim the benefit under Title 35, United States Code, section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, section 112. I acknowledge the duty to disclose material information as defined in Title 37, Code of

Federal Regulations, section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status-patented, pending, abandoned

2-D

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3-D

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Inventor's signature: Divya C

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4-D

Full name of second joint inventor, if any: Ashok Madurappa

Inventor's signature:

Date: 15th JAN 02

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